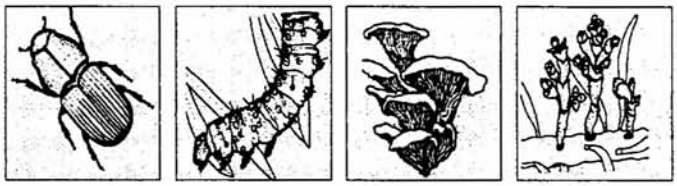
Forest Health Protection



Report 99-10 June 1999

FUNGAL COLONIZATION OF RESIDUAL CONIFER SEEDLING ROOTS IN SOIL- USDA FOREST SERVICE LUCKY PEAK NURSERY BOISE, IDAHO

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ABSTRACT

Residual conifer roots from previous seedling crops were sampled at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho for extent of fungal colonization by potential pathogens and antagonists. Samples at the beginning of the second growing season yielded higher levels of *Fusarium* (primarily *F. oxysporum)* than those at the end of the first growing season. Different nursery fields had different levels of *Fusarium* root colonization. *Fusarium oxysporum* was also common within rhizosphere soil adjacent to residual roots. Roots left in soil can provide an important source of *Fusarium* inoculum for infection of subsequent conifer seedling crops. Interactions between *Fusarium* inoculum and microbial ecology are discussed in relation to disease management.

INTRODUCTION

Diseases caused by soil-borne fungi are important limiting factors in production of bareroot forest seedlings in nurseries. Fungi capable of eliciting disease on seedlings are often considered facultative parasite, i.e., they may reside in and colonize organic matter saprophytically throughout much of their life cycle, but are also capable of invading and parasitizing live host tissues (Gerik and Huisman 1985; James and others 1991; Park 1959; Taylor and Parkinson 1961). Between periods when suitable hosts are available, many of these potential pathogens remain viable as resting spores either on pieces of colonized organic matter or within soil (Bloomberg 1966; Gordon and others 1989; Oritsejafor and Adeniji 1990; Park 1959). When susceptible hosts are present, resting spores may germinate, producing viable fungal mycelium which penetrates root epidermal cells and colonizes root cortex and vascular tissues in the process of inciting disease (Bloomberg 1976, 1979).

Several sources of organic matter commonly occur in forest nursery soil. Important sources include amended materials (sawdust, composts) and residual roots left from previous seedling crops and produced from horizontal and vertical root pruning. Root undercutting and pruning are usually performed periodically during the second seedling growing season to stimulate production of fibrous roots which makes lifting and planting easier.

Root diseases in forest nurseries are typically controlled by pre-plant soil fumigation with general biocides that kill most soil microorganisms (Boone 1988; James 1989). However, soil fumigation is expensive and one of the most efficaceous

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fumigants (methyl bromide) will no longer be available for use in the future due to environmental concerns (Evans and Greczy 1995; James and others 1993). Therefore, growers are looking for alternatives to soil fumigation to control soil-borne pests. One promising technique is fallowing fields with periodic soil cultivation for at least 1 year prior to sowing (Hansen and others 1990; James and Beall 1999; James and others 1996; Stone and others 1997). Without susceptible host material, pathogen levels tend to decrease and may stabilize over time (Bloomberg 1965; Park 1959). However, when susceptible seedling crops or suitable organic matter are introduced, pathogen levels can rise quickly (James and others 1996; Oritsejafor and Adeniji 1990; Stone and others 1997). One major concern with soil organic matter is the potential for pathogenic fungi to colonize this material, expanding inoculum density, and increasing disease potential on succeeding seedling crops (Hansen and others 1990). Therefore, an evaluation was conducted to determine importance of conifer roots from previous seedling crops as substrates for selected fungi, including potential plant pathogens and fungi potentially antagonistic toward pathogens.

MATERIALS AND METHODS

Two sets of samples were collected to determine quantitative colonization of residual seedling roots in three fields (1, 8, and 14) at the USDA Forest Service Lucky Peak Nursery, Boise, Idaho. Ponderosa pine (*Pinus ponderosa* Laws.) was being grown in fields 1 and 14; lodgepole pine *(Pinus contorta* Dougl.) was located in field 8. The first sample was collected at the end of the first growing season (October) between seedling beds in the three fields. For this sample, 11 randomly located soil samples were collected; each sample was taken to a depth of about 15 cm. Samples were kept refrigerated prior to analysis.

Our technique for quantifying fungal root infection was modified from several previously described procedures (Bloomberg 1976; Gordon and others 1989; James 1998; Larkin and others 1993a). Each soil sample was sieved (2 mm sieve) to separate organic matter, including residual seedling roots, from soil particles. Roots with attached rhizosphere soil were placed in beakers of sterile, distilled water and agitated to remove as much soil as possible. Roots were then surface sterilized in a 10% bleach (0.525% aqueous sodium hypochlorite) solution, rinsed in sterile water, blotted dry and placed directly on a selective agar medium for *Fusarium* spp. (Komada 1975). Lengths of roots placed on media were measured. Plates were incubated for 7-10 days under diurnal cycles of cool, fluorescent light at about 24°C. Fungi emerging from roots were identified to genus and selected isolates of *Fusarium* were transferred to carnation leaf (Fisher and others 1982) and potato dextrose agar for identification (Nelson and others 1983). Number of colonies of selected fungal species emerging from all root segments were determined. Mean colonization rates were calculated as the number of colonies/100 ml of sampled root. Presence of selected fungi in the rhizosphere was determined by dispensing and spreading evenly 1 ml of the water soil solution from root washings directly on Komada's media. Identification and number of fungal colonies were determined as described above.

A second root sample was taken at the start of the second growing season (May). Soil was collected between seedling beds near plots established to monitor seedling responses to dazomet fumigation (James and Beall 1999). Twenty-five soil samples were collected in each field and processed as described above, except that detached roots were dissected into several 3-5 mm pieces prior to surface sterilization. Root pieces were placed on Komada's medium and the number of pieces colonized by selected fungi determined. Data were expressed as the percentage of sampled root pieces colonized by particular fungi.

RESULTS

Residual conifer roots in nursery soil produced from previous seedling crops were readily colonized by *Fusarium* and *Trichoderma* spp. by the end of the first growing season of the subsequent seedling crop (table 1). Colonization by four different *Fusarium* spp. occurred: *F. oxysporum* Schlecht., *F. solani* (Mart.) Appel & Wollenw., *F. sporotrichioides* Sherb., and *F. acuminatum* Ell. & Ev. *Fusarium oxysporum* was the most common fungal colonizer of sampled roots. *Trichoderma* spp. were isolated less frequently, and

Cylindrocarpon spp. were only occasionally isolated from detached roots.

Assays of rhizosphere soil from the first group of sampled roots also yielded high levels of *F. oxysporum* (table 2). One other *Fusarium* species not isolated from roots, *F. sambucinum* Fuckel, was found in rhizosphere soil. Levels of *F. oxysporum* were fairly consistent in rhizosphere soil.

The second group of residual root samples (tables 3, 4, and 5), taken at the beginning of the second growing season, yielded higher levels of *Fusarium* colonization than the first samples. Colonization rates of *Fusarium* were highest in field 1 (table 3), intermediate in field 8 (table 4) and lowest in field 14 (table 5). *Fusarium oxsyporum* was again the most common *Fusarium* spp. colonizing roots; the same *Fusarium* species encountered in previous samples were often isolated. In addition, *F. sambucinum* colonized roots in fields 8 and 14 and *F. avenaceum* (Fr.) Sacc. was detected on some roots in fields 1 and 8.

Ratios of *Trichoderma* to *Fusarium* root colonization may be helpful in determining interactions between these two groups of important soil fungi (James and Beall 1999; James and others 1996). The highest ratios (greater *Trichoderma* relative to *Fusarium* colonization) were found in fields 1 and 8 at the beginning of the second growing season (tables 3 and 4, respectively). Greater averages of *Fusarium* than *Trichoderma* root colonization were detected at the end of the first growing season (table 1) and in field 14 at the beginning of the second growing season (table 5).

Table 1. Colonization of residual roots from previous seedling crops by selected fungi at the end of the first growing season - USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Sample	Root	Colonization Rate[2]						
Number	Length	FOXY	FSOL	FSPO	FACU	ALL	CYL	TRI
1	220	1.36	0	0	0	1.36	0	0.45
2	257	1.56	0	0	0	1.56	0.39	2.72
3	276	3.62	0	1.09	0	4.71	0	1.45
4	279	2.87	0	0	0	2.87	0.72	2.51
5	256	0.36	0.78	0	0	1.17	0	1.56
6	245	0.82	0.41	0	0	1.22	0.82	2.45
7	258	1.55	0	0	0	1.55	1.16	1.16
8	250	2.00	0	2.40	0	4.40	0.40	1.60
9	259	4.23	0	0.39	0.39	5.02	0	2.70
10	281	2.49	0.71	0	0	3.20	0.36	1.07
11	212	4.72	0	0.47	0	5.19	0	0
Ave.	279.3	2.33	0.18	0.39	0.04	2.94	0.36	1.65
Percent	-	79.3	6.1	13.4	1.2	100	-	-

[1] Samples were randomly collected from between seedling beds in fields 1, 8 and 14. Last row designates percent of *Fusarium* isolates.

[2] Number of colonies/100 mm of sampled root; root length in mm; FOXY = *Fusarium oxysporum;*

FSOL = *F. solani;* FSPO = *F. sporotrichioides;* FACU = *F. acuminatum*; ALL = all *Fusarium* species; CYL = *Cylindrocarpon* spp.; TRI = *Trichoderma* spp.

DISCUSSION

Soil-borne fungi capable of eliciting damping-off and root diseases of conifer seedlings in forest nurseries are well adapted to the soil environment and their populations respond to presence of organic matter and/or susceptible host plants. *Fusarium* spp. are usually the most important potential root pathogens in many forest nurseries (Bloomberg 1976, 1979; Edmonds and Heather 1973; Hartley 1921; James and others 1991). *Fusarium* usually exists in soil or organic debris as resting spores (chamydospores and sclerotia), which readily form following colonization and nutrient depletion of plant material (Burgess and others 1989; Griffin 1981; Hammeschlag and Linderman 1975; Hsu and Lockwood 1973; Opennorth and Endo 1985). These resting spores may remain viable in soil for long time periods, although they will eventually be killed, primarily by other soil microorganisms. Not all *Fusarium* species are equally capable of causing seedling diseases. The most common *Fusarium* species associated with diseased conifer seedlings is *F. oxysporum* (Bloomberg 1976, 1979; Enebak and others 1990; Gifford 1911; James and others 1991). This species has an extremely wide host range, infecting many different types of plants including conifers (James and others 1991; Kistler 1997; Matuo and Chiba 1966). Isolates of *F. oxysporum* may cause either vascular wilt diseases or root and stem decay (Kistler 1997; Lock 1973; Martyn and others 1989). The taxon designated *Fusarium oxysporum* really includes many different fungi that have similar morphological features (Correll and others 1986b; Puhalla 1985) but wide genetic variability (Correll and others 1986b; Elmer and Stephens 1989; Gordon and Okamoto 1991; Kistler 1997; Larkin and others 1996). Pathogenic strains are usually fairly host specific; strains infecting specific plant hosts are designated as a form species (formae specialis) because of their pathogenic adaptation to specific hosts (Baayen and others 1989; Gerlagh and Blok 1988; Nelson and others 1983). Pathogenic isolates infecting conifer species have been placed in the forma specialis *pini*, even though several conifer genera may be affected (Lock 1973; Matuo and Chiba 1966). However, there is some doubt whether the form species concept applies well to *F. oxysporum* strains causing conifer seedling diseases (Stone and others 1997).

Table 2. Presence of *Fusarium* spp. in rhizosphere soil of residual conifer seedling roots - USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Sample	*Fusarium* Propagules[2]		
Number	FOXY	FSAM	All *Fusarium*
1	35	0	35
2	45	0	45
3	39	0	39
4	59	0	59
5	41	1	42
Average	43.8	0.2	44.0

[1] Samples were randomly collected between seedling beds in fields 1, 8 and 14.

[2] Number of *Fusarium* colony-forming units/ml rhizosphere soil solution; FOXY = *Fusarium oxysporum;* FSAM = *F. sambucinum.*

Most isolates of *F. oxysporum* within nursery soil are non-pathogens (Hocking 1968; Nagao and others 1990; Schneider 1984; Vaartaja and Hill 1965). Within specific fields, non-pathogens usually exhibit extensive genetic variability even though they developed as asexual clones, since sexual reproduction in *F. oxysporum* is unknown (Elmer and Stephens 1989; Gordon and Okamoto 1992c; Kistler 1997). Non-pathogenic strains readily colonize roots of conifer seedlings and weeds in nurseries (Elias and others 1991; Farias and Griffin 1990). These strains are also important colonizers of soil organic matter (Brownell and Schneider 1985; Gordon and Okamoto 1992a, 1992b, 1992c; Palmer and Kommedahl 1969; Park 1959). Rapid buildup of *F. oxysporum* populations may occur when organic matter is added to nursery soil (Bloomberg 1976, 1979; Couteandier and Alabouvette 1990; French and Nielsen 1966).The fungus goes through several cycles of spore germination, colonization, and spore formation in relatively short time periods, resulting in increasing populations when sufficient food sources are present (Buxton and others 1989; Edmonds and Heather 1973). Populations on organic matter include both pathogenic and non-pathogenic strains, since both readily colonize plant material (Gerik and Huisman 1985). *Fusarium* spp. rapidly colonize organic matter and plant roots in response to chemical exudates produced by plant cells (Chi and others 1964; Kommedahl 1966). When exudates are produced, resting spores germinate and infection of the food source occurs (Buxton 1962; Farquhar and Peterson 1989; Kraft 1974). *Fusarium* spp. are most vulnerable in the vegetative stage to antagonism by other microorganisms. Therefore, *Fusarium* spp. must rapidly colonize fresh plant tissues before being adversely affected by other soil microorganisms (French and Neilsen 1960; Gerik and Huisman 1985; Palmer and Kommedahl 1969; Park 1959).

One way to control diseases caused by *F. oxysporum* is to reduce soil populations of this fungus (Haware and Neve 1982; Oritsejafor and Adeniji 1990). If populations can be kept well below about 1000 cfu/g of soil, disease problems are usually minimal (Hildebrand and Dinkel 1988).

One of the most successful ways of reducing populations is pre-plant soil fumigation with general biocides (Boone 1988; Boyd 1971; James 1989). Unless pathogens are reintroduced into fumigated fields on seed (James 1986; James and others 1991), transplants (James 1985), or soil particles from surrounding non-fumigated fields (Danielson and Davey 1969; Marois and others 1983), soil fumigation usually results in low disease levels and production of high-quality seedlings (Cordell 1982; Miller and Norris 1970). Some chemical fumigants work better than others. For example, methyl bromide is usually very effective in most nurseries (Evans and Greczy 1995; James 1989). However, dazomet, another fumigant with different toxicity characteristics, works well in some nurseries (Barnard and others 1991; Campbell and Kelpsas 1988; James and others 1996), but not in others (Carey 1995; Hoffman and Williams 1988; James and Beall 1999). *Fusarium* populations can also be reduced by fallowing fields for at least one growing season prior to sowing. Fallowing limits food availability to pathogens (Hansen and others 1990; James and others 1996; Stone and others 1997).

In some cases, *Fusarium*-caused disease levels remain low even though pathogenic strains occur in soil. These soils are classified as disease "suppressive" (Larkin and others 1993b; 1996; Schneider 1984; Park 1963). Suppressive soils contain fairly high populations of bacteria and fungi that are antagonistic toward pathogens (Park 1963; Smith 1977). They may also contain populations of non-pathogenic *F. oxysporum* strains (Larkin and others 1993b; Shishkoff and Campbell 1990); non-pathogens often compete successfully with pathogenic strains for food and infection sites (Elias and others 1991; Farias and Griffin 1990; Nagao and others 1990; Schneider 1984). Soil texture, chemical characteristics and organic matter content may also affect disease suppression (Amir and Alabouvette 1993; Bhatti and Kraft 1992; Larkin and others 1993b, 1996; van den Driessche 1963). In some cases, disease suppression is enhanced by amending soil with particular biocontrol fungi and bacteria (Beale and Pitt 1990; Sinclair and others 1975), including specific non-

pathogenic *F. oxysporum* strains (Amir and Al-abouvette 1993; Hillocks 1986).

Table 3. Colonization of residual conifer roots from previous seedling crops by *Fusarium* and *Trichoderma* spp. at the start of the second growing season (Field 1) - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Sample	Root	Colonization Rate[1]				
Number	Length[2]	FOXY	FSOL	FAVE	ALL FUS	TRI
1	160	7.50	0	0	7.50	3.75
2	170	2.35	0	0	2.35	14.12
3	90	3.33	0	0	3.33	4.44
4	90	7.78	0	0	7.78	5.55
5	85	3.53	0	0	3.53	1.18
6	75	0	0	0	0	4.00
7	100	13.00	0	0	13.00	4.00
8	175	5.71	0	0	5.71	4.00
9	155	6.45	0	0	6.45	5.81
10	260	5.38	0	0	5.38	6.15
11	70	8.57	0	0	8.57	20.00
12	100	7.00	0	0	7.00	14.00
13	130	0.77	0	0	0.77	8.46
14	95	2.10	1.05	0	3.16	0
15	80	3.75	0	0	3.75	0
16	30	3.33	0	0	3.33	10.00
17	190	0.53	2.10	0	2.63	2.10
18	95	0	0	0	0	3.16
19	180	2.22	0	0	2.22	9.44
20	160	2.50	0	0.63	3.13	5.00
21	145	4.83	0	0	4.83	1.38
22	145	0	0	0	0	13.10
23	130	0.77	0	0	0.77	3.85
24	85	3.53	0	0	3.53	4.71
25	110	4.54	0	0	4.54	1.82
Averages	118.4	4.05	0.17	0.03	4.26[3]	5.95[3]

[1] Number of colonies/100 mm of sampled root; FOXY = *Fusarium oxysporum*; FSOL = *F. solani*; FAVE = *F. avenaceum*; ALL FUS = All *Fusarium* spp.; TRI = *Trichoderma* spp.

[2] mm

[3] *Trichoderma*/*Fusarium* Ratio = 1.40.

Table 4. Colonization of residual conifer roots from previous seedling crops by *Fusarium* and *Trichoderma* spp.at the start of the second growing season (Field 8) - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Sample	Root	Colonization Rate[1]						
Number	Length	FOXY	FSOL	FAVE	FACU	FSAM	ALL	TRI
1	100	2.00	0	0	0	0	2.00	5.00
2	165	4.24	0	0	0	0	4.24	1.82
3	90	2.22	0	0	0	0	2.22	3.33
4	95	0	0	0	0	0	0	0
5	200	3.50	0	0	0.50	0	4.00	15.00
6	225	5.33	0	0	0	0	5.33	8.89
7	75	4.00	0	0	0	0	4.00	0
8	75	2.67	0	0	0	0	2.67	16.00
9	310	6.78	0	0	0	0	6.78	10.00
10	270	5.55	0	0	0	0	5.55	10.37
11	280	1.07	0	0	0	0	1.07	7.50
12	80	2.50	0	0	0	0	2.50	0
13	160	0	0	1.88	0	0	1.88	10.00
14	130	4.61	0	0	0	0	4.61	6.15
15	90	0	0	0	0	0	0	8.89
16	165	1.21	0	0	0	0	1.21	0
17	170	0.59	0	0	0	0	0.59	7.06
18	145	0	0	0	0	0	0	11.03
19	75	1.33	1.33	0	0	0	2.67	2.67
20	30	6.67	0	0	0	0	6.67	10.00
21	120	10.00	0	0	0	0	10.00	4.17
22	120	3.33	0	0	0	0	3.33	5.00
23	105	3.81	0	0	2.86	0	6.67	3.81
24	120	2.50	0	0	0	0.83	3.33	5.83
25	115	4.35	0	0	0	0	4.35	1.74
Ave.	140.4	3.30	0.03	0.08	0.11	0.03	3.56[2]	6.89[2]

[1] Number of colonies/100 mm of sampled root; FOXY = *Fusarium oxysporum*; FSOL = *F. solani*; FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSAM = *F. sambucinum*; ALL = all *Fusarium* spp.; TRI = *Trichoderma* spp.; root length in mm.

[2] *Trichoderma/Fusarium* ratio = 1.94.

Table 5. Colonization of residual conifer roots from previous seedling crops by *Fusarium* and *Trichoderma* spp. at the start of the second growing season (Field 14) - USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Sample	Root	Colonization Rate[1]				
Number	Length[2]	FOXY	FACU	FSAM	ALL FUS	TRI
1	100	17.00	0	0	17.00	2.00
2	245	2.04	0	0	2.04	2.04
3	110	0.91	0	0	0.91	3.64
4	240	4.17	0	0	4.17	5.42
5	65	0	0	0	0	4.61
6	160	5.63	0	0	5.63	0
7	80	0	0	0	0	0
8	90	0	0	0	0	0
9	85	0	0	0	0	4.71
10	250	2.40	0	0	2.40	1.20
11	105	1.90	0	1.90	3.81	2.86
12	155	0.64	0	0.64	1.29	3.23
13	30	0	0	3.33	3.33	0
14	130	0.77	0	0	0.77	6.15
15	165	2.42	0	0	2.42	1.82
16	75	1.33	0	0	1.33	1.33
17	35	0	0	0	0	0
18	50	0	0	0	0	0
19	90	7.78	0	0	7.78	0
20	60	3.33	0	0	3.33	0
21	135	0	0	0	0	0
22	70	4.29	0	0	4.29	0
23	70	1.43	0	0	1.43	0
24	20	0	5.0	0	5.0	0
25	60	10.00	0	0	10.00	0
Average	107.0	2.84	0.04	0.15	3.03[3]	2.02[3]

[1] Number of colonies/100 mm of sampled root; FOXY = *Fusarium oxysporum*; FACU = *F. acuminatum*; FSAM = *F. sambucinum*; ALL FUS = all *Fusarium* spp.; TRI = *Trichoderma* spp.

[2] mm

[3] *Trichoderma/Fusarium* ratio = 0.67.

We found that roots from previous seedling crops provided important substrates for *Fusarium* spp. potentially pathogenic to conifer seedlings. Levels of *Fusarium* root colonization at the Lucky Peak Nursery approximated that from live seedlings at another nursery (James 1998). Increasing pathogen populations and higher disease levels may be expected if high numbers of residual roots occur in soils. In the high clay soils at the nursery, extensive root breakage may occur when soil moisture is high during lifting. Root pruning and undercutting also adds root fragments to soil.

Potential impacts of residual roots on future seedling crops may be ameliorated by soil fumigation, fallowing fields longer, amending with biocontrol agents, or physical removal of roots. Machinery designed for cleaning beaches in recreation areas may effectively remove roots and other organic matter from soil (W. Littke, personal communication). However, this option may not be available or cost effective for practical use. More intensive soil cultivation to break up roots and periodically bring soil and organic debris to the surface may reduce pathogen populations by physical destruction. Without soil fumigation, closer monitoring of pathogen populations and more innovative ways of disease control will be necessary in the future.

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